

Bactericidal activities of fifty medicinal plants methanolic extracts against *Pseudomonas syringae* pv. *syringae*

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ABSTRACT

In this current investigation biocontrol of bacterial pathogen was achieved by methanolic extracts of fifty different medicinal plant extracts via agar well diffusion method. Concerning bacterial pathogens affecting sorghum plants, *Pseudomonas syringae* causal agent of seedling diseases of sorghum, is one of the most spread, in greenhouse and in open field sorghum crops in the world. Natural extracts with microbicidal activity of fifty different medicinal plants were studied to improve crop development. The inhibitory effects of methanol extracts of these plants with different MICs were tested against bacterial phytopathogen, *P. syringae*. It is gram negative rod shaped bacteria belonging to the family Pseudomonadaceae. It causes seedling diseases, bacterial blight of lilac; tomato speck disease and bacterial canker of cherry etc, the antimicrobial activities of the methanol extracts of most of the medicinal plants have demonstrated the inhibition effects on *P. syringae*. Except the following four plants such as *R. communis*, *T. pumila*, *T. tinctoria* and *T. procumbens* were not exhibited antimicrobial activity against *P. syringae*. Among the selected fifty plants 92% of plants given remarkable bioactivity where as only the 08% of plants did not give antimicrobial activity against *P. syringae*.

Key words: *Pseudomonas syringae*, Methanolic extracts, Bactericidal activity.

INTRODUCTION

Pseudomonas syringae is a rod shaped Gram-negative plant pathogenic bacteria that can be characterized by its inability to properly utilize arginine, because it lacks the assistance of the arginine dihydrolase system. In hot dry weather *P. syringae* populations often dramatically decrease in relative size and abundance. Once again, there are exceptions to these generalizations, depending on the particular strain¹. Not all strains of *P. syringae* are pathogenic. The strains that are pathogenic

cause disease on their hosts through the release of toxins and cell wall degrading enzymes. The result of disease occurring in the plant is actually a function of the pathogen population size and not just the presence of a pathogen.

A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of new drugs. Medicinal plants are rich sources of ecologically developed secondary metabolites, which are potential

remedies for different ailments. Extreme interest in plants with microbicidal activity has revived as a result of current problems such as resistance associated with the use of antibiotics obtained from microorganisms. The main advantage of natural agents that they do not enhance the antibiotic resistance, a phenomenon commonly encountered with the long-term use of synthetic antibiotics. The use of phytochemicals as natural antimicrobial agents commonly called "biocides" is gaining popularity². There is growing interest in correlating phytochemicals constituents of plant with its pharmacological activity³. There has been growing interest in the investigation of the natural products from plants for the discovery of new antimicrobial agents as an alternative route for the synthetic chemicals, side effects of which are always in question. It has been reported that the higher plants have shown to be a potential source for the new antimicrobial agents⁴. Based on the above information the authors selected and collected fifty different medicinal plants from coastal region of Andhra Pradesh. They were extracted by using Soxhlet apparatus and have been evaluated microbicidal activity against *P. syringe*.

MATERIAL AND METHODS

The plant materials (Table No. 1) were collected from coastal area of Andhra Pradesh. They were identified taxonomically with the help of faculty from Andhra University, Visakhapatnam.

Preparation of Methanolic extracts

The plant material denoted in Table 1 were dried under shade, powdered by using blender and stored in air tight bottle to avoid missing of active compounds. The powdered material was extracted with methanol by hot extraction process using Soxhlet apparatus. The extract was evaporated and the dried in vacuum desiccators and stored in refrigerator until used.

Microorganism employed

Pseudomonas syringe an important phytopathogen which causes seedling diseases in sorghum, citrus blast, tomato speck disease, is a gram negative rod shaped bacteria. The bacteria was grown in the nutrient broth at 37p C and maintained on nutrient agar slants at 4p C until used.

To evaluate antibacterial study nutrient agar medium was used. Pure culture of bacterium was removed from agar slants and transferred to nutrient broth, incubated at 37p C for 24h. The turbidity was adjusted to that of standard level by adding sterile nutrient broth.

Antimicrobial analysis

The methanolic extracts of fifty different plant extracts were screened for antimicrobial activity by agar well diffusion method⁵ with cork borer of size 6.0mm. The overnight cultures grown in nutrient broth was used for inoculation of nutrient agar plates. An aliquot (0.02ml) of inoculums was introduced to molten and cooled at 45p C nutrient agar and placed on petri dish by pour plate technique. After solidification the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method 0.05ml of methanolic extracts of fifty different plant extracts were introduced serially after successful completion of one plant analysis. Incubation period of 24-48h at 37p C was maintained for observation of antimicrobial activity of plant extracts. The antimicrobial activity was evaluated by qualifying zones of inhibition of bacterial growth surrounding the plant extracts. The complete antimicrobial analysis was carried out under strict aseptic conditions and results were tabulated in table 2.

RESULTS

The initial examination of microbicidal activity of fifty different methanolic extracts was reported in table 2. Among the selected fifty plants forty six plants given remarkable bioactivity where as only the four plants such as *Recinus communis*, *Tephrosia pumila*, *Tephrosia tinctoria* and *Tridax procumbent* were not exhibited antimicrobial activity against *P. syringe*. The biocide potentiality exhibited by fifty plants with variable concentration not with similar concentration of the extract. According to this criterion plants may be grouped as A and B and called them as high potential plants and moderate potential plants respectively. The plants given activity up to 50mg/ml and 55-100mg/ml concentrations were treated as group A and B respectively. The plants *Bridilia montana*, *Melia azedarach* and *Scoparia dulcis* were given bioactivity up to 50mg/ml concentration. Among

Table 1: List of Investigated Medicinal Plants

Botanical Name	Parts used	Uses / Ailments treated
<i>Acacia farnesiana</i> (L.) Willd	Bark, roots	Astringent, Demulcent, Poultice, Stomachic.
<i>Acalypha indica</i> Linn.	Aerial parts	Skin diseases, Ulcers Bronchitis, Head ache, Snake bite
<i>Acanthus ilicifolius</i> Linn.	Leaf extract	Relieve rheumatism
<i>Adenocalymma alliaceum</i> (Lam.)	Leaves	Astringent
<i>Adhatoda vasica</i> Nees.	Leaves, whole plant	Cough chronic bronchitis, rheumatism, asthma and asthma.
<i>Andrographis paniculata</i> Nees.	Whole plant	Anti-biotic, anti-viral, anti-parasitic and immune system stimulant.
<i>Avicennia officinalis</i> L.	Seed	Relieving ulcers
<i>Boerhaavia diffusa</i> Linn.	Whole plant	Scabies, myalgia, aphrodisiac
<i>Bridelia montana</i> (Roxb.) Willd	Leaf	Stomach pains, sore eyes and headaches.
<i>Cassia occidentalis</i> Linn.	Whole plant	Boils, Spasm. Hysteria, Whooping cough
<i>Catharanthus roseus</i> Linn.	Leaves and roots	<u>Anti-mitotic</u> and <u>Anti-microtubule</u> agents
<i>Centella asiatica</i> Linn.	Whole Plant	Diuretic, treatment of leprosy, use as brain tonic and stimulates hair growth.
<i>Cleome viscosa</i> Linn.	Leaves and seeds	<i>Anthelmintic, carminative, diaphoretic and rubefacient.</i>
<i>Coleus forskohlii</i> (Willd.).	Roots	Treat heart and lung diseases, intestinal spasms, insomnia and convulsions. Antispasmodic.
<i>Coriandrum sativum</i> Linn.	Fruits	Colic, Laxative, Blood purifier, Indigestion, sore throat
<i>Derris scandens</i> (Roxb.) Benth	Stem	Arthritis, Anti-inflammatory
<i>Eichhornia crassipes</i> (C.Mart.)	Whole plant	Biomass, soil reclamation
<i>Emblica officinalis</i> Gaertn.	Fruit	Aperient, Carminative, Diuretic, Aphrodisiac, Laxative, Astringent and Refrigerant.
<i>Gmelina arborea</i> Linn.	Roots	Gonorrhoea, catarrh of bladder, cough, cleaning the ulcers, insanity, epilepsy, fevers, indigestion, nerve tonic.
<i>Gynandropsis gynandra</i> (L.)	Leaf	Anti-irritant
<i>Hildegardia populifolia</i> (Roxb.)	Stem bark	Dog bite, Malaria.
<i>Hiptage benghalensis</i> (L.) Kurz.	Leaves and bark	Insecticidal, cough, inflammation; skin diseases and leprosy
<i>Holarrhena antidysenterica</i> Foxh.	Bark and seeds	Dysentery, piles, leprosy, colic, dyspepsia, chronic chest complaints, spleen diseases, jaundice, bilious, calculi
<i>Hyptis suaveolens</i> (L.) Poit.	Leaves	Antispasmodic, antirheumatic and antisporific
<i>Kyllinga nemoralis</i> Rottb.	Whole Plant	Promotes action of liver, and relief prunitus

<i>Lantana camara</i> Linn.	Whole Plant	Antidote to snake venom, Malaria, wounds cuts ulcers, Eczema, Tumours
<i>Marraya Koenigii</i> (L.) Spreng.	Leaves	Skin diseases, Heminthiasis, Hyperdipsia, Pruritus, etc.
<i>Melia azedarach</i> L.	Leaves, Seed Flower, Oil, Vermifuge	Insecticide, Astringent, Tonic and Antispetic. It possesses anti diabetic, anti bacterial and anti viral
<i>Mimosa pudica</i> Linn.	Whole Plant	Menorrhagia, piles, Skin wounds Diarrhoea, Hydrocele, Whooping cough, Filiriasis
<i>Moringa heterophylla</i> L.	Roots,Seeds,	Antibiotic Anti-inflammatory and Diabetes
<i>Muntinga calabria</i> Linn.	Leaves	Antiseptic
<i>Ocimum sanctum</i> Linn.	Leaves, Seeds	Malaria, bronchitis, colds, fevers, absorption, arthritis.
<i>Peltophorum pterocarpum</i> (DC.)	Whole plant	Reclamation
<i>Phyllanthus niruri</i> L.	Leaves	Jaundice, Diabetes
<i>Plumeria rubra</i> Linn. rubefacient.	Leaves	Ulcers, leprosy, inflammations,
<i>Pongamia pinnata</i> (L.) Pierre. rheumatic and leprous sores	Bark, seeds	Anti malarial , skin disease,
<i>Ricinus communis</i> Linn.	Leaves	Jaundice, sores,
<i>Salvadora persic</i> , Linn.	Roots	Antimicrobial and dental diseases
<i>Scoparia dulcis</i> Linn.	Whole plant	Used for upper respiratory problems, congestion, menstrual disorders, fever, wounds and hemorrhoids
<i>Sesbania grandiflora</i> (L.)	Flowers	Gonorrhoea and for curing infection of the cornea.
<i>Strychnos - nux - vomica</i> Linn.	Seeds	Cholera, chronic wounds, Ulcers, paralysis, Diabetes
<i>Suaeda maritima</i> (L.) Dumort.	Whole plant	Bioremediation
<i>Tephrosia pumila</i> (Lamk.) Persoon.	Root	Rheumatism, fevers, pulmonary problems, bladder disorders, Coughing, hair loss, and reproductive disorders
<i>Tephrosia tinctoria</i> Pers.	Root	Antisiphilitic
<i>Tephrosia villosa</i> (L.) Pers.	Root,Leaves,Bark	Anthelmintic, alexiteric, leprosy, ulcers, antipyretic, cures diseases of liver, spleen, heart, blood, asthma etc.
<i>Terminalia chebula</i> Retz.	Fruit	Antimicrobial, digestive problems, mouthwash/gargle, astringent, and douche for vaginitis.
<i>Tinospora cordifolia</i> (Willd.)	Stem	Analgesic and anti-inflammatory.
<i>Tridax procumbens</i> Linn.	Whole plant	Antimicrobial, Anti-oxidant and Anti-inflammatory,
<i>Vitex pentaphyllal</i> Linn.	Aerial parts	Foetid discharges, Febrifuge Rheumatism affections, catarrhal
<i>Withania somnifera</i> (L.) Dunal	Leaves	Sore eyes, Febrifuge, ulcers Cure sterility of women sedative

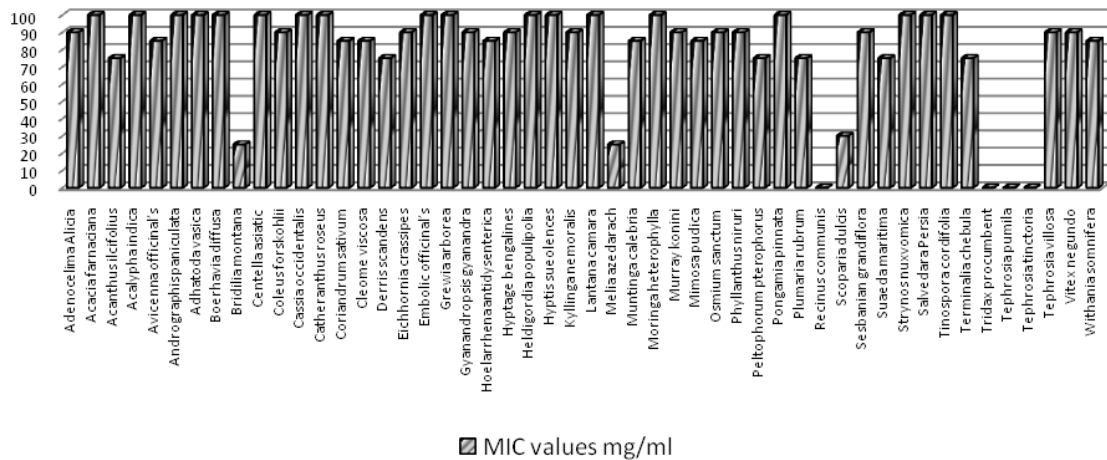


Table 2: MIC analysis of fifty different plant methanolic extracts on *Pseudomonas syringe*

them the high potential was exhibited by *Bridelia montana* and *Melia azedarach* at 25mg/ml conc. Most of the tested plants have reported moderate activity with 55-100mg/ml conc. Among them *Acanthus ilicifolius*, *Derris scandens*, *Peltophorum pterophorus*, *Plumaria rubrum*, *Suaeda maritima* and *Terminalia chebula* were shown nice moderate activity with 75mg/ml conc. And good moderate activity also exhibited by *Avicenna officinalis*, *Coriandrum sativum*, *Cleome viscosa*, *Hoelarrhena antidysenterica*, *Muntinga calabura*, and *Withania somnifera* with 85mg/ml concentration.

DISCUSSION

The plants which ecologically synthesis and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils, minerals and vitamins, they possess medicinal properties. Higher and aromatics plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts⁶. In previous studies, it was reported that methanol was a better solvent

for the consistent extraction of antimicrobial substances from medicinal plants compared to other solvents such as water, chloroform and hexane^{7,8}. Therefore, methanol was used for plant extraction in this study. From this present investigation we found some plants which are having very useful antimicrobial properties against *P.syringe*. Meryem et al⁹, reported that antimicrobial activity of *V. georgicum* against *P. syringe* with 300µg/disk concentration. In our present examination the extracts of *B. montana* and *M. azedarach* have shown micobicial activity with 500µg/well concentration. Therefore, this result may suggest that Methanolic extracts of screened plants would be helpful in treating citrus blast, tomato speck disease in plants. In particular, the authors recommend that the methanolic extract of *Bridelia montana*, *Melia azedarach* and *Terminalia chebula* can be used as potent biocides to treat the plant disease caused by *Pseudomonas syringe*. The observed findings suggested the further work on all the selected plants to evaluate their potential for use as antibacterial to treat bacterial diseases in humans.

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